

ANNOTATION

RECENT PROGRESS WITH THE TREPONEMAL IMMOBILIZATION TEST*

The new approach to the serology of syphilis made possible by Nelson's development of a practical method of demonstrating treponemal immobilizing antibodies has been quickly followed up by workers in many other countries and a considerable literature has already accumulated.

It was apparent from the outset that the technique presented many difficulties, and it is understandable that much attention has been paid to technical aspects of the test. A conference of those working with the test was held under the auspices of the Federal Security Agency in Washington in February, 1952. At this meeting practical difficulties which had been encountered were fully and frankly discussed, and improvements in technique suggested.

One major inconvenience of the test has been that the serologist is made a slave to his rabbits, because of the necessity of harvesting the testes within 48 hrs of the development of a clinical orchitis. If harvested before they are ripe, the testes yield insufficient treponemes, and if left beyond this time limit, the organisms may be sensitized to such a degree by antibody produced by the rabbit that they cannot safely be used for the test. Chorprenning and others (1952) have described a method by which testes can be removed at the time of election and preserved at -65 to -55°C . for periods of at least 3 months. Treponemes extracted from these frozen testes can be used satisfactorily for the test as well as for infecting other rabbits, but the incubation period tends to be longer than when organisms from fresh testes are used. Inhibition of sensitization by irradiation of the rabbit with x rays or by treatment with nitrogen mustards has been used successfully, but animals so treated have an appreciable mortality and although their testes are very rich in treponemes, the appearance of a clinically detectable orchitis is retarded.

Cain (1953) using a suspension of dead treponemes has developed a treponemal agglutination test which is read under the dark-ground microscope.

The treponemal suspension is stable for at least 9 weeks at 4°C ., and the results obtained on 122 sera agreed very closely with those given by the immobilization test. This may be a development of great potential importance, as it may make the test more suitable for routine use. Complete inhibition of sensitization of treponemes is, however, imperative, and this necessitates treating the rabbits used as a source of treponemes with either x rays or nitrogen mustard with the drawbacks inherent in these procedures.

A considerable amount of attention has been devoted to the medium used to extract the treponemes from the infected testes. Even minute traces of oxygen as impurity in the $\text{N}_2\text{-CO}_2$ atmosphere in which the test is carried out may kill the organism. Removal of oxygen by passing the gas over red hot copper or by increasing the thioglycollate content of the medium has been used with success by Reyn (Symposium, 1953). Portnoy and others (1953a, b) have also reported better survival and more reproducible results when the thioglycollate content of the medium was increased to five times the amount present in Nelson's medium and more complement was added to the test mixtures. Saurino (1953) proposed a simplified medium in which thioglycollate and glutathione were omitted but extra serum ultrafiltrate or inactivated serum was added. While these simplifications may be welcomed from a practical viewpoint, the ultimate aim should be a medium of chemically defined composition capable of reproduction in any laboratory. The introduction of such substances as serum is, from this point of view, a retrograde step, and when assessing the efficiency of any proposed modification of the medium it must be remembered that during the process of extraction of the testis, tissue juice of unknown composition is added to the medium and probably contributes substances promoting survival.

One of the curious features of the test is the length of time necessary for sensitization of the treponemes by the immobilizing antibody. It is

* Received for publication October 14, 1953.

now recognized that when tests are read after 18 hrs' incubation at 35°C. as in Nelson's original method, the reaction may not have proceeded to completion. With such long incubation periods much non-specific destruction of complement occurs, and pre-sensitization techniques have been used to avoid this. In these, complement is added after a preliminary incubation period during which union of treponemes with antibody occurs, immobilization only taking place after the complement has been added. The amount of complement used has a marked effect on the sensitivity of the test, and some workers have attempted to overcome this by using a definite dosage of complement in haemolytic units, rather than a fixed volume of guinea-pig serum. Recent work on the kinetic aspects of the test has been reviewed by Daguet (Symposium, 1953).

It will be readily appreciated that comparison of results is difficult when laboratories are using a diversity of techniques, especially with quantitative tests. The World Health Organization has taken a welcome step in sponsoring the distribution of a pooled positive serum to the various laboratories undertaking the test. The inclusion of a quantitative test on such a serum in each batch of tests set up may make possible a comparison of sensitivity levels in different laboratories. At the same time it gives the individual worker an indication of day-to-day variations in the sensitivity of his test. Some dissatisfaction with the reproducibility of the test is evident in recent papers (Portnoy and others, 1953 a, b; Olansky and others, 1953; Saurino, 1953; Harris and others 1953), and further work on this important aspect seems to be needed.

The results of tests on large numbers of a wide variety of sera have now been published (Durel and others, 1952; Miller and others, 1952 a, b; Chacko, 1953; Miller and Smith, 1953). A recent meeting of the *Filiale Marseillaise de la Société Française de Dermatologie et de Syphilographie* was devoted to the various aspects of the T.P.I. test. The papers given at this meeting have recently been published in book form (Symposium, 1953) and are of great interest as reflecting the extensive experience of French workers with the test. Opinions regarding the value of the immobilization test in its applications to the practical serology are now becoming more clear-cut. It seems likely that it will find its most extensive use as a verification procedure with sera thought to give non-specific reactions with the standard reagin tests, particularly those of the chronic type. Comparative studies on sera giving non-specific reactions have

been made between the immobilization test and the Kahn verification test by Harrell (1953) and with the Neurath euglobulin inhibition test by Roy and others (1953). In both cases the results given by the immobilization test were considered to be more reliable. The test seems to have no place as a diagnostic procedure in primary and secondary syphilis, and to have only confirmatory value in patients with symptomatic syphilis in whom the reagin tests are positive. It may also give useful help in the investigation of patients presenting lesions which may be due to syphilis, although the reagin tests are negative—in some cases of aortic regurgitation, for example. Its place for the present is that of an ancillary to the standard reagin tests, and in this role it will have an important part to play provided that clinicians realize that it has limitations and is still primarily a research procedure.

The immobilization test has given particularly interesting results in cases classed as "latent syphilis" on the basis of repeatedly positive reagin tests in the absence of supporting clinical evidence or history of infection. Moore and Mohr (1952 a, b) have reported that out of 300 patients who were sero-positive with standard reagin tests for 6 months or longer and showed no other evidence of infection, 43 per cent. gave negative immobilization tests. A similar figure was obtained by Nelson (1953) from a study of 496 untreated United States servicemen who had given two or more positive or doubtful reagin tests (mainly the Standard Kahn test) and who showed no clinical evidence or history of syphilitic infection. If, as the evidence so far produced suggests, a negative immobilization test means that these positive reagin tests were non-specific in nature, it seems possible that in Great Britain also, this type of patient may present a diagnostic problem of considerable magnitude. Moore and Mohr (1952b), investigating a number of their patients who were thought to be "chronic biologically false positive reactors", found that a considerable number of them were suffering from diseases of the collagen group. Thus a negative immobilization test, while possibly exonerating the patient from the suspicion of syphilis, may also act as a pointer to the presence of a potentially more serious disease.

In its present form, the technical difficulties and expense of operating the test will necessarily restrict its performance to specialized laboratories, and some selection of sera for testing will unfortunately be necessary. Since such a promising tool for investigating the serology of syphilis has been developed, it is to be hoped that the opportunities it offers will now be grasped in Great Britain

and that facilities for research into both the academic and practical aspects of the immobilizing antibody will be made available.

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BOOK REVIEWS

Management of Chancroid, Granuloma Inguinale, and Lymphogranuloma Venereum in General Practice. By Robert B. Greenblatt, M.D., C.M., with the collaboration of E. R. Pund, M.D., E. S. Sanderson, Ph.D., M.D., Richard Torpin, M.D., and R. B. Dienst, Ph.D. 1953. Pp. 66, 29 figs, bibl. Public Health Service Publications No. 255, U.S. Department of Health, Education, and Welfare (V.D. Division), Government Printing Office, Washington, D.C., U.S.A. 30 cts.

This small paper-backed book is packed with valuable information on these less common but extremely interesting venereal diseases. It can be recommended as an instructive booklet which can be read in conjunction with articles on the same subjects published in the "British Encyclopaedia of Medical Practice", 2nd edition, 1950-52. The wealth of illustration is of particular value to those whose clinical experience of these diseases is restricted by geographical and climatic considerations.

Interested physicians can obtain a copy gratis from the Chief, Division of Venereal Disease, U.S. Public Health Service, Washington, 25, D.C. A. O. F. R.

Progress in Venereology. By R. R. Willcox. 1953. Pp. vii + 191, 36 figs. Heinemann, London. 21s.

This book, published in April, 1953, presents progress in venereology since the second world war. It is a clear, concise and remarkably coherent survey of the literature published between 1946 and 1952, summarizing, with references, over 1,700 articles and reports. It covers all aspects of the venereal diseases and associated conditions, including incidence, laboratory diagnosis and experimental work, control measures, and all the treponematoses. The tables and graphs are of interest and the clinical photographs are all good.

The period reviewed is one of major development in diagnosis and treatment of the individual, and in planning and mounting mass attacks on the trepone-

matoses in countries where these are endemic in epidemic proportions. The literature of the period is correspondingly large and exciting and this book provides a fair summary and easy reference to it. The book is only of value to the venereologist and others with a sound basic knowledge of the subject, but to such readers, and in particular to the teacher and author, it will be invaluable. The author is to be congratulated most warmly on completing a difficult task so successfully and the publishers deserve credit for providing such good value for a guinea. We hope that the present book is only the forerunner of a series of similar surveys of progress in venereology. S. M. L.

Atlas of Medical Mycology. By Emma Sadler Moss and Albert Louis McQuown. 1953. Pp. ix + 246, 248 illus. Bailliere, Tindall and Cox, London. 61s. 6d.

The authors state that this book is the outcome of lectures and demonstrations given as part of a training programme for residents in pathology, clinical residents, medical students, and medical technologists. The aim is to present a concise basic plan of action for clinicians and clinical pathologists who are confronted with diagnostic problems concerning fungus diseases.

To make a success of such an ambitious plan in the space of 246 pages is exceedingly difficult. Nevertheless, the authors have produced an excellent Atlas of Medical Mycology illustrated by some 248 photographs, most of which are very informative and beautifully reproduced.

As might be expected, the text concerning the clinical features of the diseases described and the treatment thereof is somewhat sketchy, whilst the inclusion of a glossary with phonetic spelling seems unnecessary. At the same time, this book should prove to be most useful to the laboratory worker, containing as it does, not only the means by which the various fungi of medical interest may be identified but also information on the contaminants which are apt to make this type of work so difficult. I. N. O. P.